

Mechanisms of Morphine Enhancement of Spontaneous Seizure Activity

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BACKGROUND: High-dose opioid therapy can precipitate seizures; however, the mechanism of such a dangerous adverse effect remains poorly understood. The aim of our study was to determine whether the neuroexcitatory activity of high-dose morphine is mediated by selective stimulation of opioid receptors.

METHODS: Mice hippocampi were resected intact and bathed in low magnesium artificial cerebrospinal fluid to induce spontaneous seizure-like events recorded from CA1 neurons.

RESULTS: Application of morphine had a biphasic effect on the recorded spontaneous seizure-like events. In a low concentration (10 μ M), morphine depressed electrographic seizure activity. Higher morphine concentrations (30 and 100 μ M) enhanced seizure activity in an apparent dose-dependent manner. Naloxone, a nonselective opiate antagonist blocked the proconvulsant action of morphine. Selective μ and κ opiate receptor agonists and antagonists enhanced and suppressed the spontaneous seizure activity, respectively. On the contrary, δ opioid receptor ligands did not have an effect.

CONCLUSIONS: The proseizure effect of morphine is mediated through selective stimulation of μ and κ opiate receptors but not the activation of the δ receptor system. The observed dose-dependent mechanism of morphine neuroexcitation underscores careful adjustment and individualized opioid dosing in the clinical setting.

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Chronic severe pain afflicts more than 6% of the general population, and high-dose opioids are the mainstay of treatment (1). As research and education regarding the importance of opioid use in chronic severe pain continue to improve, the number of patients treated with high-dose opioids increases correspondingly, as does the incidence of opioid-related adverse effects (2). Among the most curious and dangerous of these adverse effects is neuroexcitation. Myoclonus and seizures have been reported with high doses of morphine (3,4), hydromorphone (4,5), meperidine (6), fentanyl (7), diamorphine (8), and after oral (9), IV (3,4,7), epidural (5), or intrathecal (8,10) administration. Indeed, the recognized neuroexcitatory effects of high-dose short-acting opioids, such as fentanyl, alfentanil, and remifentanyl, are routinely harnessed

to induce controlled electroencephalographic seizures during epilepsy surgery at our institution (11,12).

The mechanism, and thus treatment, of opioid-related neuroexcitation remains unclear. Previous animal investigations have suggested that the proconvulsant action of morphine is mediated through the 3-glucuronide morphine metabolite (13) or nitric oxide produced by constitutive nonspecific nitric oxide synthase (14). Other studies have proposed that multiple receptor systems are involved in triggering opioid-induced seizures, including opioid, adrenergic, and glutamatergic receptors (15,16), or by opioid antagonism of inhibitory γ -aminobutyric acid (GABA)ergic neurotransmission (17). Finally, some animal reports have implicated muscarinic and cannabinoid receptors in morphine enhancement of seizures (18,19).

A neuroexcitatory mechanism involving direct activation of opioid receptors has yet to be demonstrated definitively in the intact hippocampus. The intact *in vitro* hippocampal model allows precise control of the tissue environment and the direct application of selective receptor ligands without the involvement of different pharmacokinetic factors that can influence the access of such molecules to their respective site of action. Furthermore, the intact hippocampus maintains the neural pathways directly involved in seizure initiation and propagation, as well as the physiologic neuronal rhythms (20). The present study aimed to determine whether the neuroexcitatory effect of

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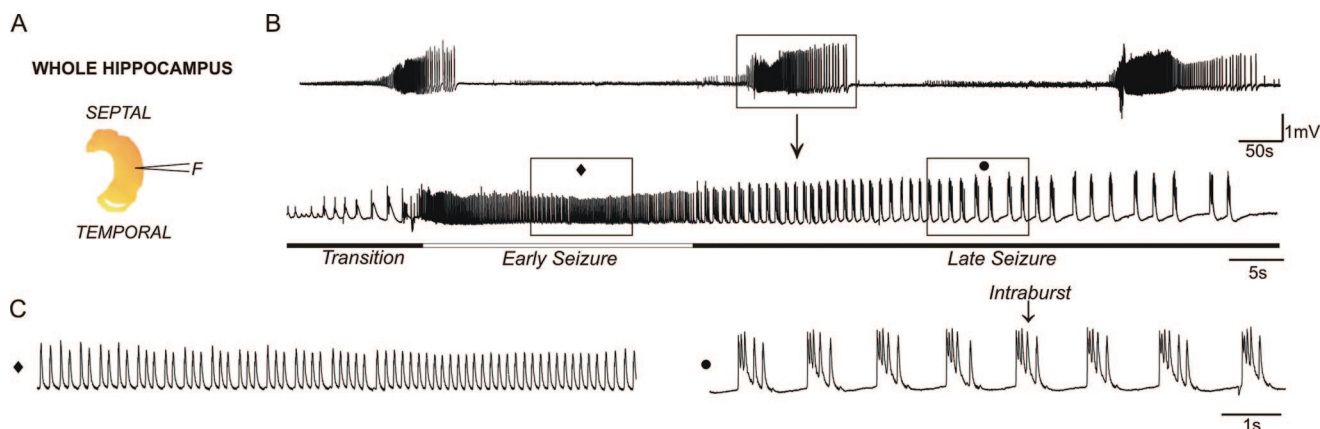


Figure 1. Seizure-like events recorded in the intact hippocampus during perfusion with low Mg^{2+} artificial cerebrospinal fluid (ACSF). (A) A diagram illustrating the intact whole mouse hippocampus that was maintained *in vitro*. The septal and temporal poles are noted. The position of the recording electrode (F) is shown which corresponds to the location of the CA1 pyramidal cell layer of the hippocampus. (B) Electrophysiologic recordings showing spontaneous seizure-like events in the intact hippocampus induced by low Mg^{2+} ACSF perfusion. An expanded time scale of a single seizure-like event (box) shows three distinct components. First component is a transition period with low frequency spikes followed by an early and late seizure phases. (C) Further expansion of the time scale shows different components of the early and late seizure-like events. The late seizure component consists of slow late burst and fast intraburst frequencies.

morphine is mediated through direct stimulation of specific opioid receptors. We hypothesize that morphine enhancement of seizure activity is mediated through selective stimulation of opioid receptors in the intact hippocampus.

METHODS

Dissection

The University Health Network Animal Care Committee review board approved this animal study protocol. C57/BL mice at 8th to 25th postnatal day were anesthetized with halothane and decapitated in accordance with the Canadian Animal Care Guidelines. The brain was carefully removed and bathed in ice-cold 2–5°C oxygenated (95% O_2 , 5% CO_2) standard artificial cerebrospinal fluid (ACSF) containing the following: 123 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 , and 15 mM glucose. After removal of the cerebellum and the separation of the hemispheres at the midsagittal line, spatulas were used to carefully remove the brainstem and the midbrain region. The hippocampus was then resected by gently sliding the spatula ventral to the corpus callosum, making the first insertion at the septal hippocampal region. The septum was left intact and served as an anatomical marker for the location of the septal and temporal hippocampus regions (Fig. 1A). Hippocampi were bathed in oxygenated ACSF at room temperature for a minimum of 1.5 h before subsequent transfer into the recording chamber (21).

Recording Chamber Environment

The intact hippocampus was maintained in a recording chamber at a temperature of $34^\circ C \pm 0.5^\circ C$, continuously perfused with oxygenated standard ACSF and secured by micropins. Humidified warmed

oxygen flowed over the solution in the recording chamber to decrease the evaporation of oxygen from the perfusate. Solutions entered the chamber at a flow rate of 5 mL/min, refilling the volume of the 0.6 mL chamber with fresh solution eight times per minute. Seizure-like electrographic activity was induced by perfusing the tissue with low- Mg^{2+} ACSF containing the following: 123 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 0.25 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 , and 15 mM glucose.

Electrophysiology

Recordings were performed from the CA1 pyramidal neuronal layer of the hippocampus by positioning the hippocampus DG-CA3 region dependent on nylon mesh and the CA1 region faced upwards. A Grass S44 (Grass Medical Instruments, West Warwick, RI) stimulator with bipolar electrode was used to stimulate Schaffer collaterals (1–15 V, 0.1 ms) and extracellular glass electrodes containing 150 mM NaCl that were positioned at locations yielding maximal evoked and spontaneous field potential amplitudes in the CA1 stratum pyramidale. Input/output relations were recorded with stimulating pulses of 10 different intensities, evoking subthreshold to suprathreshold population responses. All single-site recordings of seizure-like events were obtained from the middle of the hippocampus (Fig. 1A), and filtered with 1 kHz low-pass filter unless otherwise stated, amplified, and recorded at 2 kHz sampling rate with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). The signals were digitized using the 16-bit data acquisition system Digidata 1322A (Axon Instruments, Foster City, CA). Data visualization was achieved with Clampfit 8.2 (Axon Instruments, Foster City, CA). All results are shown as mean values \pm SEM.

Table 1. Naloxone Actions on Spontaneous Electrographic Seizure Activity Recorded from the Whole Hippocampus *In Vitro*

| | Naloxone 5 μ M ($n = 5$) | | | Naloxone 10 μ M ($n = 5$) | | |
|---------------------------|--------------------------------|-----------------|------------------|---------------------------------|-------------------|------------------|
| | Control | Effect | Recovery | Control | Effect | Recovery |
| Seizure duration (s) | 98.78 \pm 9.7 | 102 \pm 10.23 | 105.1 \pm 8.86 | 118.1 \pm 8.36 | 73.56* \pm 3.46 | 94.61 \pm 7.67 |
| Late burst frequency (Hz) | 1.1 \pm 0.15 | 1.2 \pm 0.17 | 1.03 \pm 0.09 | 1.1 \pm 0.15 | 1.2 \pm 0.17 | 1.03 \pm 0.09 |
| Intraburst frequency (Hz) | 23.1 \pm 2.14 | 24 \pm 3.2 | 22.2 \pm 2.45 | 13.1 \pm 0.45 | 0.00 | 11.1 \pm 0.45 |

* Statistical significance from control ($P < 0.05$).

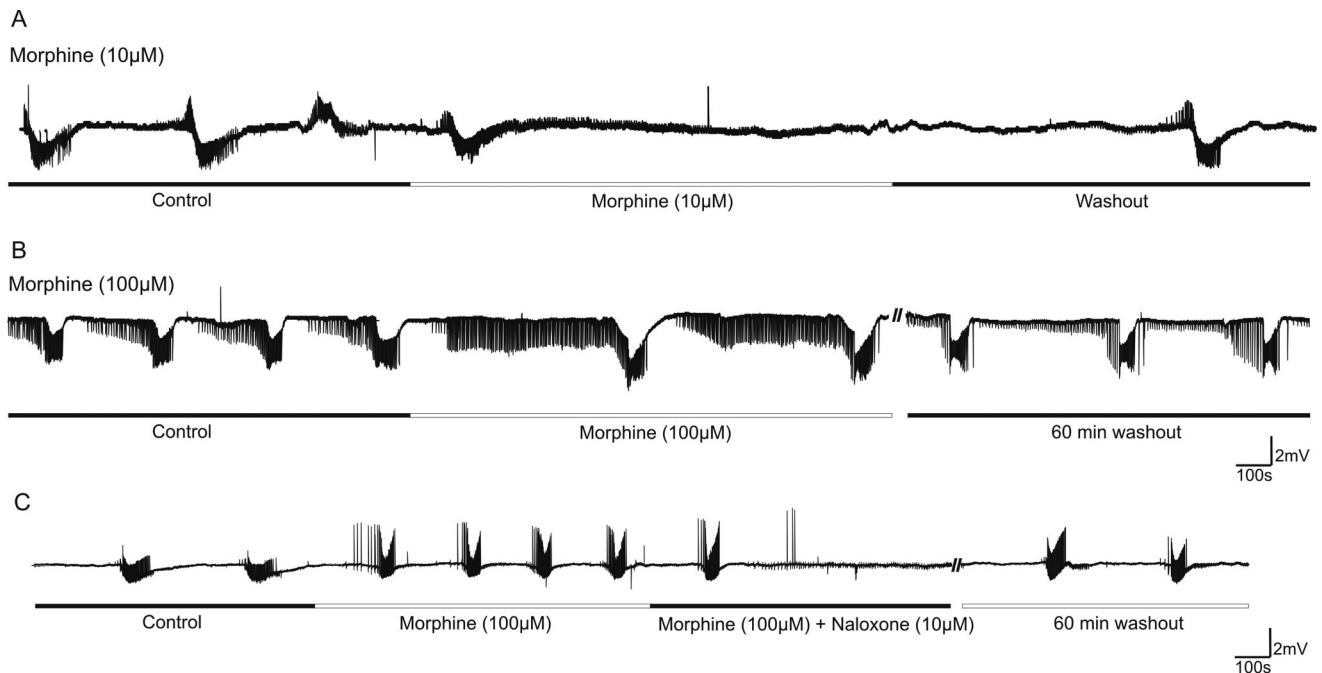


Figure 2. Morphine actions on seizure-like events in the intact hippocampus *in vitro*. Morphine in a low concentration 10 μ M depressed seizure-like events in the intact hippocampus *in vitro* (A) whereas a higher concentration 100 μ M enhanced seizure activity (B). The effects of morphine 100 μ M were blocked by the concomitant application of naloxone 5 μ M (C).

Drugs

All drugs used in the present study were obtained from Sigma-Aldrich (St. Louis, MO), except morphine sulfate purchased from Abbott (Toronto, Canada). Drugs were dissolved in standard ACSF or low Mg^{2+} ACSF to designated concentrations as follows: morphine (10, 30, and 100 μ M); selective μ -opioid receptor agonist, DAMGO (10 μ M) and antagonist, B-FNA (10 μ M); κ -opioid receptor agonist, Dyn-A (10 μ M) and antagonist, nor-BNI (10 μ M); and δ -opioid receptor agonist, DPDPE (10 μ M) and antagonist, NTI (10 μ M).

Statistical Analysis

The electrographic seizure activity was quantified by determining mean \pm SEM of seizure duration, late seizure frequency, and intraburst frequency (Figs. 1B and C). The seizure duration is the time elapsed between the start of the early phase and the termination of the late phase not including the transition, i.e., prodromal phase (Fig. 1B). Where applicable, data were analyzed using one-way and repeated measures ANOVA and paired and unpaired *t*-test. Statistical significance was considered at a $P < 0.05$.

RESULTS

Morphine Actions

Control experiments were performed to determine the consistency and stability of the seizure activity induced by perfusing the intact hippocampus with low Mg^{2+} ACSF. In each of 10 different hippocampi, the recurrent seizure patterns were consistent and did not change their electromorphologic appearance. Recurrent seizures had a mean duration of 87 ± 5 s, and there were 2 ± 0.65 seizures per 10 min. Usually, the recurrent seizure events started approximately 6–12 min after the administration of low Mg^{2+} ACSF and lasted for approximately 95–120 min in a consistent and stable manner. Single maximal postsynaptic population spikes were evoked by stimulating the Schaefer collateral to monitor the viability of the hippocampi. Experiments were terminated if there was a deterioration of the population spike amplitude below 75% relative to their initial values. A single seizure consisted of an early and a late phase (Fig. 1B). The early phase consisted of a series of single spikes with a mean frequency of 1 Hz. The late phase comprised of multiple bursts with a mean burst frequency of 1.3 Hz.

The intraburst frequency was defined as the number of spikes per second contained in a single burst occurring during the late phase of a seizure (Fig. 1C).

Because naloxone is considered a nonspecific opiate antagonist for morphine, we performed experiments to elucidate the action of this drug on the seizure activity of the epilepsy model used in this study. Naloxone 5 μM applied to hippocampi perfused with low Mg^{2+} ACSF had no effect on the spontaneous electrographic seizures. However, naloxone 10 μM had a statistically significant effect in attenuating seizure duration (Table 1).

The application of morphine to intact hippocampi exhibiting seizure activity revealed a biphasic effect. Morphine 10 μM decreased the duration of the seizure activity and the number of recurrent seizure events per 10 min (Figs. 2A and 3A). However, the late burst and intraburst frequencies were not affected (Figs. 3B and C). These effects were completely reversed after a washout period of about 20 min. The addition of naloxone 5 μM to the perfusate did not reverse such inhibitory actions of morphine. When higher concentrations of morphine (30 and 100 μM) were applied, seizure activity was enhanced in duration in a dose-dependent manner (Figs. 2B and 3A). Naloxone 5 μM blocked such enhancement in seizure activity (Figs. 2C and 3A). Meanwhile, higher morphine concentrations did not significantly change the early phase frequency but depressed the late bursting and intraburst frequencies (Figs. 3B and C). Higher morphine concentrations (30 and 100 μM) administered to the hippocampi perfused with standard ACSF did not cause any seizure activity. Moreover, the enhancement of seizure activity in hippocampi perfused with low Mg^{2+} ACSF containing morphine was not sustained when the same hippocampi were subsequently perfused with standard ACSF containing morphine.

Effects of Selective Opioid Ligands

To address the question of which opioid receptor was responsible for mediating the proconvulsive action of morphine, we applied selective opioid agonists and antagonists to hippocampi exhibiting seizure activity when perfused with low Mg^{2+} ACSF (Fig. 4; Table 2). The μ and κ opiate receptor selective agonists, DAMGO 10 μM and Dyn-A 10 μM , respectively, enhanced seizure activity as evidenced by the increase in seizure duration and number of recurrent seizure events per 10-min intervals. Similar to the higher morphine concentrations (30 and 100 μM), the μ and κ opiate receptor agonists decreased the late burst and intraburst frequencies. On the contrary, the μ and κ receptor antagonists, i.e., B-FNA and nor-BNI, respectively, depressed seizure discharges in seizing hippocampi perfused with low Mg^{2+} ACSF. Such depression of seizure discharges was in the form of a decrease in seizure duration and the conversion of the seizure into a state of stable firing rate (Figs. 4A and B). The latter effect might have been due to the

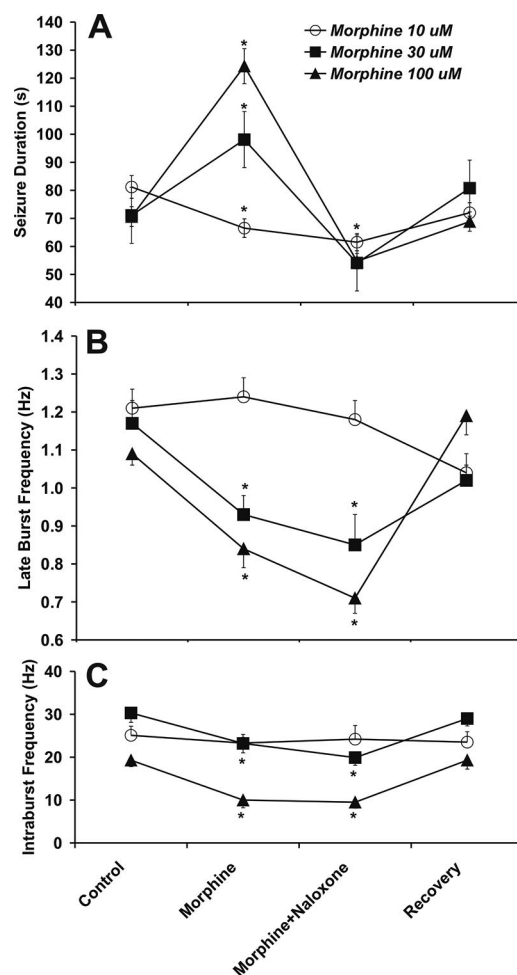


Figure 3. Dose-dependent effects of morphine on hippocampus seizure-like events. Morphine 10 μM decreased and morphine 30 and 100 μM increased the durations of seizure-like events recorded from the intact hippocampus in a dose-dependent manner (A). Morphine 30 and 100 μM suppressed the late burst and intraburst frequency components of the seizure-like events (B and C). Naloxone 5 μM reversed the effects of morphine 30 and 100 μM on the duration but not on the late burst and intraburst frequencies (A–C). Error bars represent (\pm SEM). Each morphine concentration was applied to at least six intact hippocampi. *Statistical significance from control ($P < 0.05$).

agonist-induced shift of the neuronal activities from a disinhibited “down-state” that leads to the seizures to an active “up-state,” i.e., a stable increased firing rate. Neither selective δ receptor agonist DPDPE 10 μM nor its antagonist NTI 10 μM had any significant effect on the electrographic seizure events.

DISCUSSION

The most important finding of the present study is that the neuroexcitatory action of morphine is mediated through selective stimulation of the μ and κ opiate receptor subtypes. This study is the first to demonstrate that a high concentration of morphine can enhance spontaneous seizure activity in an apparent dose-dependent manner in the intact hippocampus maintained *in vitro*. Such action had been

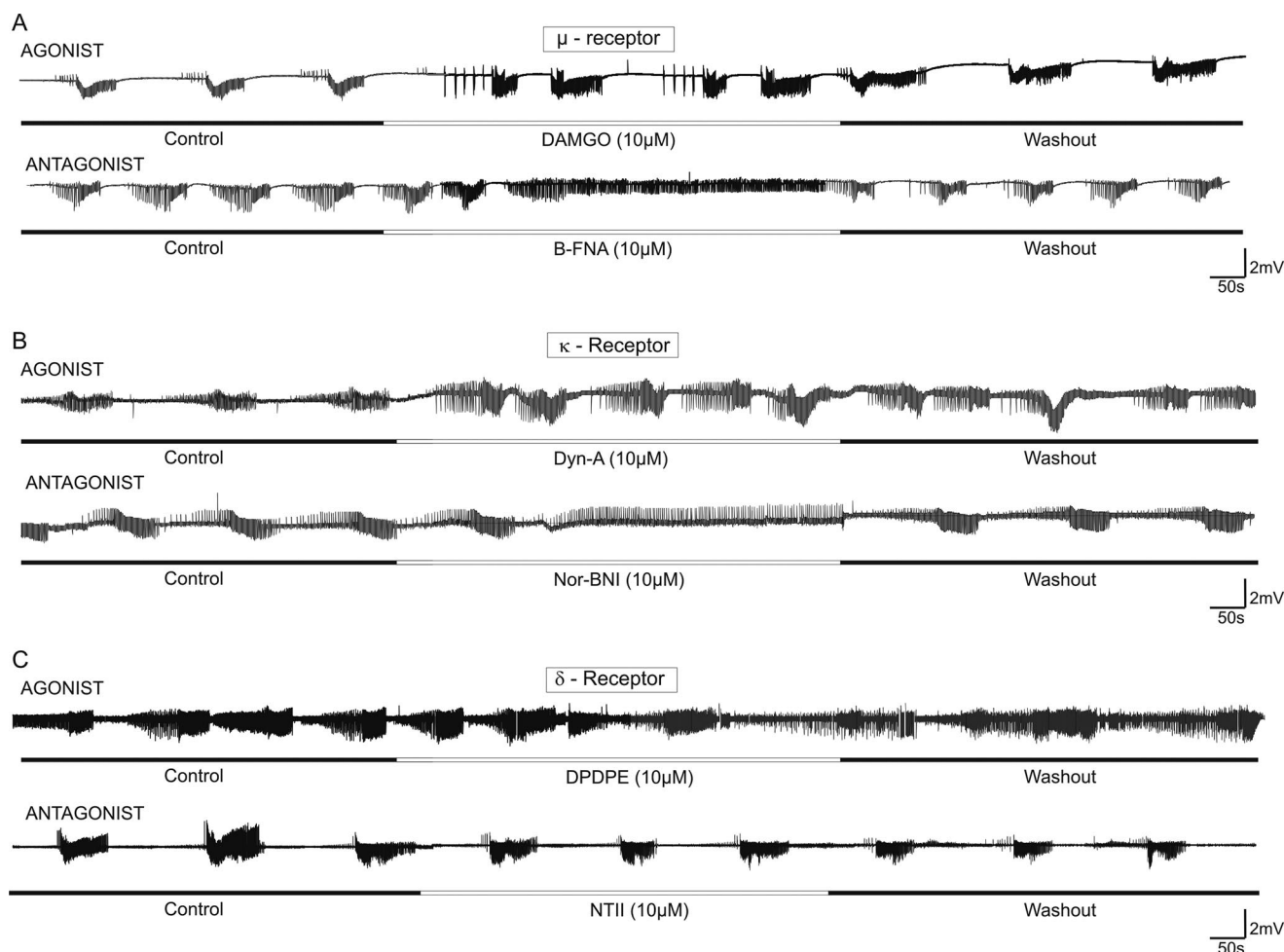


Figure 4. Actions of selective opioid receptor agonists and antagonists on hippocampal seizure-like events. μ (A) and κ (B) selective opioid receptor agonists and antagonists enhanced and suppressed spontaneous seizure-like events in the intact hippocampus, respectively. On the contrary, δ receptor agonists and antagonists (C) did not have any effect on hippocampus seizure activity.

Table 2. Effects of Opiate Receptor Ligands^a on Spontaneous Electrographic Seizures Recorded from the Intact Hippocampus *In Vitro*

| | μ -Receptor | | κ -Receptor | | δ -Receptor | |
|---|-----------------|--------------------|--------------------|----------------------|--------------------|------------------|
| | Agonist (DAMGO) | Antagonist (B-FNA) | Agonist (Dyn-A) | Antagonist (Nor BNI) | Agonist (DPDPE) | Antagonist (NTI) |
| Seizure duration (% control) ^b | 129.59* ± 13.8 | 84.18* ± 10.1 | 137.487* ± 15.12 | 68.74* ± 6.87 | 82.03 ± 10.66 | 109.91 ± 12.64 |
| Late burst frequency (% control) ^b | 57.26* ± 7.9 | 113.13 ± 14.8 | 91.16* ± 13.6 | 89.01 ± 9.97 | 92.68 ± 12.98 | 103.68 ± 16.59 |
| Intraburst frequency (% control) ^b | 68.10* ± 1.36 | 92.62 ± 3.34 | 61.46* ± 2.46 | 128.10 ± 48 | 103.74 ± 3.32 | 105.49 ± 5.27 |

^a Each ligand was applied to at least 6 intact hippocampi that developed seizure like-events when perfused with low Mg²⁺ artificial cerebrospinal fluid.

^b % Control = [measurement during ligand application/control measurement] × 100.

* Statistical significance from control (*P* < 0.05).

demonstrated in whole-animal epilepsy models when morphine at higher doses was administered intraperitoneally, IV, or intracerebroventricularly (22,23). This study also confirms that morphine has a biphasic effect, as reported in previous whole-animal studies (23,24); that is, a low morphine concentration demonstrated an antiseizure effect whereas higher morphine concentrations enhanced spontaneous seizure activity.

The present investigations used an *in vitro* low Mg²⁺ model of hippocampal epilepsy so as to examine the possible mechanisms of morphine-induced seizures. Low Mg²⁺ leads to neuronal hyperexcitability that is conducive for seizure generation by decreasing membrane surface charge screening, decreasing Mg²⁺ sensitive Ca²⁺ channel block and decreasing the Mg²⁺ blockade of N-methyl-D-aspartate receptors. This

implies that different strategies can be used to treat opioid-induced enhancement of seizure activities. It is true that high concentrations of morphine did not elicit seizure activities in the present study when the Mg^{2+} concentrations were normal. However, our conclusions from the low Mg^{2+} model can be relevant to those instances when morphine induces seizure activities in the clinical situation (2–4). The low Mg^{2+} model of epilepsy showed seizure-like discharges similar to the progression of electroencephalographic abnormalities in patients with partial complex seizures and partial status epilepticus (22). It is also well recognized that a low serum Mg^{2+} concentration can cause seizures, and IV Mg^{2+} administration can prevent and control convulsions associated with eclampsia. Hence, it is reasonable to assume that the findings and conclusions of the present study can have relevance to various clinical situations.

The antiseizure effect of morphine observed in this study was similar to previously reported findings in whole-animal models of epilepsy (23,24). The mechanism of this effect in our model is probably not related to the direct stimulation of specific opioid receptors because adding naloxone 5 μ M to the perfusate with morphine 10 μ M did not reverse the antiseizure effect. It is possible that the mechanism of such action is mediated through inhibitory effects on glutamatergic excitatory pathways and increasing GABAergic activity. Although the antiseizure effect might be clinically insignificant, its occurrence indicates that our intact isolated hippocampal epilepsy model is sensitive enough to experimentally simulate the biphasic neuroexcitatory actions of morphine. The clinical implications of this effect can be expressed in a historical context, as opioids in the past had been used to abort intractable seizures in epilepsy patients (25).

Morphine-enhanced seizure activity was depicted by an apparent dose-dependent increase in the duration of ictal events. However, we also observed a simultaneous decrement in the ictal late burst and intraburst frequencies. Late burst and intraburst frequencies reflect hypersynchronous behavior of the neuronal networks involved in initiating and maintaining seizure activity (26). The morphine-induced depression of both the late burst and intraburst frequencies of the seizure-like events were probably due to multiple morphine effects at the cellular level that should be further investigated to identify their pathophysiologic significance.

Our results validate the hypothesis that the pro-seizure effect of morphine is mediated through selective μ and κ opioid receptor stimulation, but not activation of δ receptor subtypes. This is because spontaneous seizure activity was enhanced by specific μ and κ agonists, i.e., DAMGO and Dyn-A, respectively, and suppressed by μ and κ antagonists, i.e., B-FNA and nor-BNI, respectively. We can only speculate on the “downstream” mechanisms by which μ and κ -opioid receptor stimulation enhances spontaneous seizure

activity. Stimulation of the μ and κ -receptor subtypes can lead to a state of disinhibition in CA1 pyramidal neurons similar to that described in a subpopulation of periaqueductal gray neuronal elements (27). The disinhibitory effect may be mediated by decreasing the recurrent inhibition of the CA1 pyramidal cells through suppression of inhibitory GABAergic interneurons (28). The latter leads to enhancement of the recorded seizure-like events in the intact hippocampus. Suppression of GABAergic interneurons may be produced by presynaptic mechanisms that decrease GABA release. Accordingly, Vaughan et al. (27) reported that selective μ and κ -receptor agonists can reduce the amplitudes of evoked inhibitory postsynaptic currents with a simultaneous decrement in the rate of spontaneous miniature inhibitory postsynaptic currents without any effect on their amplitude distributions or kinetics in mice with intact opioid receptor subtypes. Another possibility is that the suppression of GABAergic interneurons can be a consequence of activating an inward rectifying K^+ current. This current was found to be enhanced by μ and κ agonists (27).

In conclusion, our findings indicate that the pro-seizure effect of morphine is mediated through selective stimulation of μ and κ opiate receptors. The observed dose-dependent mechanism of morphine neuroexcitation underscores the importance of careful adjustment and individualized opioid dosing in the clinical setting, and could help guide the development of therapeutic strategies to prevent and treat opioid-related seizures.

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